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CHEMORECEPTORS

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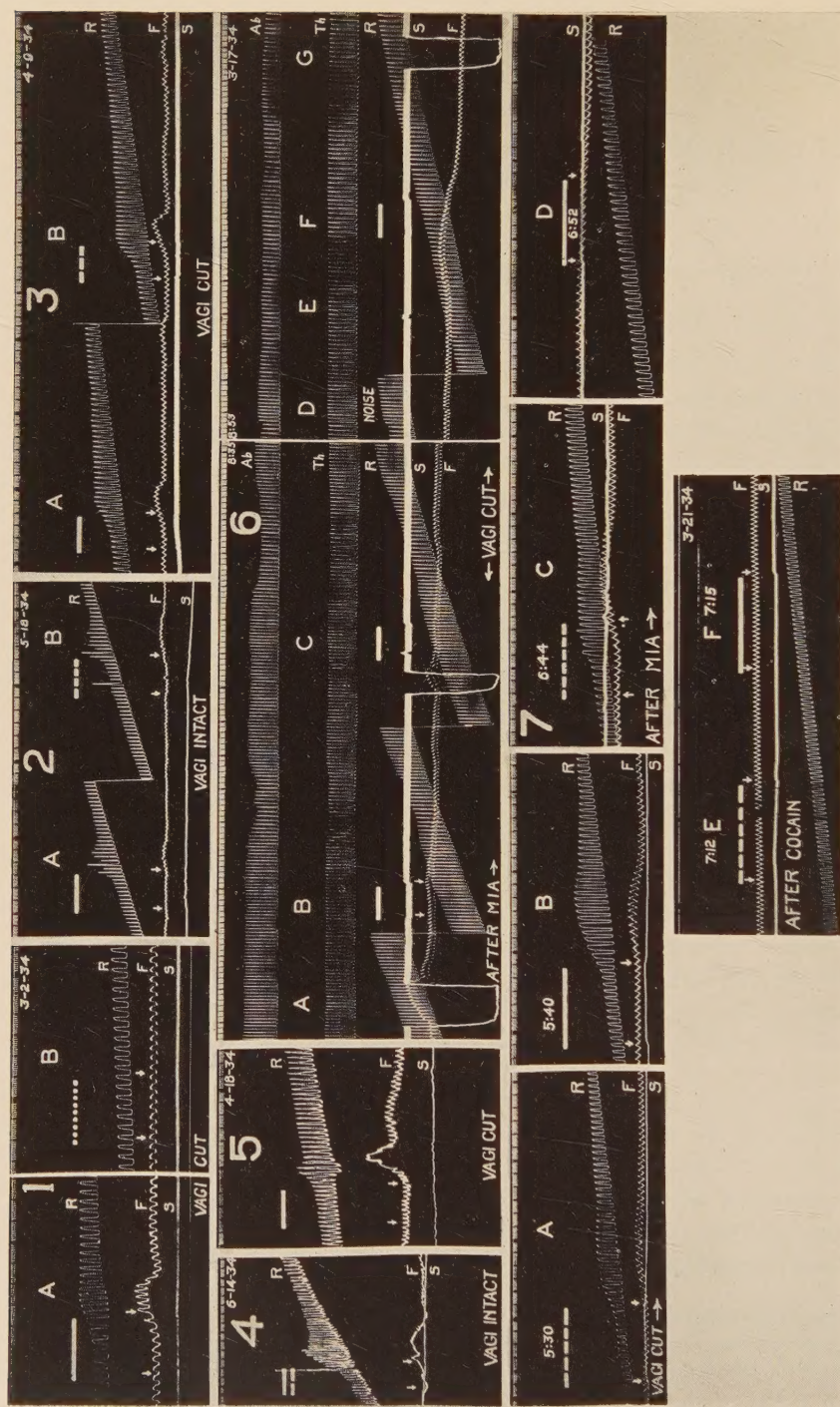
It is now well established that chemoreceptors in the carotid gland are the point of departure of important anoxemic and hypercapnic, respiratory and circulatory reflexes; but little is known definitely of the fundamental mode of activation of these chemoreceptors by the various chemicals. An attempt is made here to contribute toward the solution of this problem.

The general method of attack was a comparison of reflex responses to lack of oxygen and excess carbon dioxide restricted to the carotid segments, before and after local poisoning with mono-iodoacetic acid, in dogs anesthetized with morphine and urethane. The carotid segments were perfused continuously by use of the arrangement outlined previously (Winder, 1937). It was necessary, in vascular isolation of the carotid segments, to preserve the minute arterial connections with the carotid glands. Accessory reservoirs for shunting anoxic, hypercapnic, asphyxial (anoxic and hypercapnic), or "control" fluid into the intake of the pump were necessary. After adjustment of the levels of the reservoirs, by means of valves the desired fluid could be shunted into the circuit and through the carotid segments at will, without change in perfusion pressure. To avoid reduction in gas pressure over the reservoir fluids, each was connected with a collapsible bag containing the proper gas. The fluid was a heparinized mixture of defibrinated dog's blood and Locke's solution. That used in the continuous circuit and in the accessory reservoirs was all taken from one mixture. The waterbath insured a common temperature for all. The gas used for equilibration of the continuous and control fluids ranged from 5.56 per cent to 6.09 per cent carbon dioxide, and from 74 per cent to over 93 per cent oxygen (dry). The anoxic fluid was equilibrated with gas of effectively the same carbon dioxide content (the difference ranged from 0.00 per cent to 0.20 per cent, averaging 0.05 per cent), and less than 0.5 per cent oxygen (except in one experiment in which the oxygen percentage was 1.15). The hypercapnic fluid was equilibrated

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Figs. 1 to 7. Chemoreceptor reflexes before and after poisoning with MIA. For details see text. *R*, spirometer record of respiration (inspiration on upstroke); *Ab*, abdominal movements; *Th*, thoracic movements; *F*, femoral arterial pressure; *S*, intravenous perfusion pressure. At the uppermost level of each record is a tracing of time in seconds. — indicates anoxic perfusing fluid; indicates control perfusing fluid; - - - - - indicates hypercapnic perfusing fluid.

with gas containing 35 per cent carbon dioxide and from 62 per cent to over 64 per cent oxygen (with two exceptions, when the concentrations were 25 per cent carbon dioxide and over 72 per cent oxygen, and 30 per cent carbon dioxide and over 69 per cent oxygen, respectively). The asphyxial fluid was equilibrated with gas containing 35 per cent carbon dioxide and less than 0.5 per cent oxygen. Such extremes of anoxia, hypercapnia and asphyxia were chosen deliberately for demonstrating whatever capability of response might remain after poisoning with mono-iodoacetic acid. The control fluid was abandoned after several experiments, because in no case did its flow through the carotid segments have any reflex effect on the organism. It seemed unquestionable that effects of the reservoir fluids would be due only to their gas pressures (see fig. 1, A and B).

In general the experimental sequence was as follows: Responses to the reservoir fluids were recorded before, or before and after vagotomy. The iodoacetic acid, free or neutralized with sodium carbonate solution, was slowly injected into the circuit. The pressoreceptor reflexes were occasionally tried as in the preceding paper. After an interval, responses to the reservoir fluids were again determined.

It has for some years been an accepted theory that in the chemical control of breathing the actions of anoxemia and hypercapnia may partially converge to the common factor of acidity *within* the controlling mechanism (Gesell, 1925, 1929). The fact that the actions of these regulants diverge in other respects (Gesell and Moyer, 1935a, 1935b) is not excluded by this concept, and the established importance of the carotid gland in the chemical regulation of breathing need not materially alter the theory, inasmuch as one needs only consider a part of the chemosensory mechanism located outside the medulla. Accordingly, we arrive at an accessible though minute sensory organ from which we may hope to learn something directly concerning the mode of stimulation by anoxemia and carbon dioxide.

Since Lundsgaard's (1930a, 1930b, 1930c, 1930d) original discovery that mono-iodoacetic acid (MIA) suppresses anaerobic glycolysis in frog and mammalian muscle and in yeast this poison has been accepted as a universal suppressor of glycolysis. Under certain conditions and for a certain time, concentrations of the poison just sufficient to prevent glycolysis seem not to affect cell "respiration", or enzymatic processes other than glycolysis, to any great extent. Such indications of the potential specificity of MIA in suppressing glycolysis, together with the fact that in spite of suppression of lactic acid formation a tissue may continue for some time to perform its characteristic work, drawing upon some more primary source of energy than glycolysis, point to its possible use in a crucial

test of the hypothesized rôle of glycolysis in anoxic stimulation of sensory elements.

Henderson and Greenberg (1934) attempted such a test, but injected the poison into the intact animal rather than localizing its action, so that they were forced to use a dose (33 mgm. of MIA per kgm. body weight) inadequate for complete suppression of glycolysis (Lundsgaard, 1930a; Barrenschæen and Braun, 1931; Marenzi and Mundt, 1931; Field and Peeke, 1931; Neuss, 1931; Haldi, 1932; Haarman, 1932), in order to keep animals alive for experimentation. Indeed, before the anoxic response was tested the animals had returned to an apparently normal state. The workers argued that glycolysis was at a standstill from the observation that during gradual oxygen deprivation there was no increase in *blood* lactic acid. But on considering their statement that there was no lactic acid increase in their unpoisoned control animals, this can scarcely serve as evidence to the end intended. Further, the modern acidity theory involves changes within cells, rather than blood, and recognizes a probable lack of parallelism. In fact, there are to be found indications in the literature that poisoned mammals die before lactic acid formation in brain or muscle, for instance, is completely suppressed (Neuss, 1931; Haarman, 1932), presumably due to blockage in the central nervous system (Ledebur, 1932). For such reasons, these workers' observation that anoxic hyperpnea persisted in their "poisoned" animals cannot be considered as evidence that glycolysis is not involved in anoxic hyperpnea.

In the present experiments, where the poison was limited to the carotid segments, any desired concentration could be applied to the receptor without endangering the remainder of the reflex mechanism. The volume of fluid in the continuous circuit was known to within 15 per cent, so that the poison concentration could be estimated that closely. Three groups of experiments were performed. Six experiments were done to ascertain whether anoxic stimulation at the carotid gland is affected in any way by concentrations of the poison that are reported to be adequate to suppress glycolysis in other tissues. The vagus trunks were severed. In the first, where the highest concentration of poison was used (0.0012 for 50 minutes, and 0.0025 for 16 minutes more, before the first dilution with blood from an accessory reservoir), the pressoreceptor and the anoxic reflexes were both abolished. In the second, where the lowest concentration was used (0.00007 plus or minus 0.00003, for 74 minutes before dilution), the pressoreceptor and anoxic reflexes both continued unaffected for $1\frac{1}{2}$ hours after injection of MIA. In each of the remaining four experiments, however, where intermediate concentrations of poison were used (ranging from 0.00014 plus 0.00017 36 minutes later, with continuous dilution due to replenishing a constant fluid loss from the circuit, to 0.0007 undiluted for 20 minutes), the anoxic respiratory stimulation was entirely abolished

or so nearly so that any slight augmentation of ventilation was questionable, while the pressoreceptor reflexes persisted, though usually somewhat weakened.

If the anoxic reflex had been affected no more by the MIA than the pressoreceptor reflexes, it would have been fairly safe to conclude that glycolysis is not involved in the anoxic stimulation. The actual results of this first set of experiments, however, do not lead by themselves to a definite conclusion. The elimination of anoxic stimulation is definitely due to some action of the MIA, but one may justly ask whether it is specific elimination of glycolysis in the chemoreceptors or, for instance, surpassing the marginal concentration of MIA beyond which "respiration" of the chemoreceptors might be affected. A second series of eight experiments was therefore done, to ascertain whether the poison affects anoxic and hypercapnic responses differentially.

The poison concentrations in this series were of the same order of magnitude as those found in the first series to eliminate the normal anoxic response but not materially affect the pressoreceptor reflexes. The results of the first series were consistently confirmed in this respect. In one experiment, after poisoning neither anoxia nor hypercapnia elicited any response whatever, although the pressoreceptor reflexes had retained their original strength. In two others the anoxic and hypercapnic responses seemed abolished while the pressoreceptor reflexes were somewhat weakened. In both of these experiments increasing the perfusion pressure made it possible to elicit very slight and questionable responses with both anoxia and hypercapnia. In the remaining five experiments there were definite indications of a differential effect of the poison on anoxic and hypercapnic responses. At a time when the anoxic stimulation was entirely abolished or so very nearly so that its existence was questionable, the hypercapnic response remained, either apparently unaltered or more or less weakened. In two of these experiments the vascular isolations of the carotid segments were "complete", so that the persisting hypercapnic response could not have been an intracranial effect. In two of the three wherein small irregular branches had been left patent, external cocainization of the sinus segments completely abolished the persisting hypercapnic response, eliminating again the possibility of an intracranial effect. Records of the important stages in one experiment are shown in figure 7.

In such experiments it seems without further consideration justifiable to weight positive results much more heavily than negative, but there are circumstances suggesting the cause of the negative results of the first three experiments of this series. First, it is possible that the marginal concentration of MIA, beyond which its action becomes more generally toxic for the chemoreceptors, was surpassed. Second, certain observations indicate that the circulation through the gland was markedly retarded by

the poison; viz., an originally abrupt response to chemical stimulation of the gland commonly became more gradual and prolonged after the poisoning; and it was frequently necessary to increase the perfusion pressure to bring out a response. Work is available which bears on this retardation of circulation. Schmitt (1935) found intestinal smooth muscle to go into MIA contracture resembling that of striated muscle. In accord with this Neuss (1931) and Ueda (1934) report that MIA causes long-lasting and incompletely reversible constriction in frog and mammalian vascular beds. Dobrowolski (1933) and Irving (1934) report that MIA causes an increase in capillary permeability and edema. A contracture in the small arteries supplying the gland, coupled with edema, could cause a great disturbance in circulation and function. Such circumstances may account for considerable weakening or actual abolishment of the hypercapnic response along with the disappearance of the anoxic response, in a portion of experiments.

It seems safe to conclude from the positive results, then, that whereas the machinery of glycolysis is required for stimulation of the chemoreceptors by anoxia, it is not essential in the case of hypercapnia. We cannot go so far, however, as to claim that *no part* of the hypercapnic reaction involves glycolysis. To this point the experiments would not determine which of two possible consequences of glycolysis suppression eliminates the anoxic stimulation; viz., elimination of an actual link in the process of stimulation, or elimination of an energy source which would ordinarily be drawn upon under extreme anoxia. For that reason a third series of experiments was attempted, to determine whether hypercapnia can stimulate the poisoned chemoreceptors when they are simultaneously subjected to extreme anoxia. In addition to the anoxic and hypercapnic fluids, the "asphyxial" fluid was provided, equilibrated with the same pressure of carbon dioxide as the hypercapnic, and the same pressure of oxygen as the anoxic.

Unfortunately, in this series there were five out of seven experiments in which both the anoxic and hypercapnic, and also the asphyxic responses were abolished. In the remaining two experiments conditions were somewhat more, but not ideally, favorable for differential abolishment of the anoxic response. At stages of these two experiments where the normal anoxic stimulation was gone, the hypercapnic response persisted, though to be sure considerably weakened. More important, carbon dioxide was definitely able to stimulate under extreme anoxia (asphyxial fluid), which by itself was unable to stimulate. This is considered evidence that glycolysis is an actual link in setting up the local excitatory process by anoxia, rather than merely a necessary source of energy during the short period of anoxia. Work particularly from the laboratories of Embden and of Meyerhof indicates that MIA acts at a point in the glycolytic proc-

ess normally followed by acid intermediaries and finally lactic acid itself. It may not be premature to conclude, then, that anoxia appears to be dependent in some way upon processes of formation of acid products of glycolysis, for stimulation of the chemoreceptors of the carotid gland. It seems justifiable to assume here, as for the second series, that the lack of differential effect of MIA on anoxic and hypercapnic responses in so many experiments was due to actions of the poison other than specific suppression of glycolysis in the chemoreceptors. There seems no apparent reason, however, for the proportion of such experiments being higher than in the second series, unless it be that the MIA had undergone more decomposition.

In all three series of experiments there was usually a progressive increase in "resting" respiration beginning shortly after introduction of the MIA. The maximum increase was usually reached in about $\frac{1}{2}$ hour. Such augmented resting breathing has been observed while the pressoreceptor reflexes appeared normal, the hypercapnic reflex persisted, and the anoxic response was abolished. Carotid gland excitability appeared increased for some stimulus, though abolished for anoxia. One recalls that hyperpnea is observed on intravenous injection of the poison into intact animals (Hall and Field, 1931; Neuss, 1931; Dobrowolski, 1933; Henderson and Greenberg, 1934). Hyperpnea from general poisoning is probably not entirely a result of carotid gland poisoning, but the latter must play at least a rôle.

In all three series, at that stage of an experiment commonly characterized by augmented resting ventilation, persisting pressoreceptor and hypercapnic reflexes, and abolished anoxic stimulation, there was a tendency for anoxia to cause delayed depression of ventilation (fig. 6, B and C) at a point which would have just followed the abolished normal stimulation. Since in two experiments where anoxic depression was marked there were small vessels remaining patent between the perfused carotid segments and the systemic circulation, while in one of those wherein the anoxic depression was least evident there were no indications of patent vessels (except the minute circulation into the carotid gland), the question arose as to whether the depression resulted from the anoxic fluid's reaching the medulla. There are circumstances to eliminate this possibility. After the records shown in figure 6, B and C, were taken, respiration gradually fell "spontaneously" to about the level existing during the depressions (fig. 6, E). At that stage anoxic depression almost completely failed (fig. 6, F). The slight swell is not significant, since the animal responded even more to noises in the room (fig. 6, D). If the anoxic depressions had been intracranial effects they would have persisted in about the same percental degree when the resting level of ventilation had changed. Two alternative explanations remain; viz., stimulation of *pressoreceptors* by

the anoxic fluid, or paralysis of *chemoreceptors* responding continuously to some such stimulant as the carbon dioxide pressure of the continuous fluid. Although the former could be ruled out for normal conditions (Bronk and Stella, 1935), it must be considered under MIA poisoning. It seems probable that the other alternative explains the depressions, since the level to which respiration finally fell when the carotid gland had apparently lost most of its irritability is about the same as that during the anoxic depressions (compare fig. 6, E, with B or C).

The question arises as to whether anoxemia and hypercapnia, with their respective acid mechanisms, actually stimulate a common set of receptors, or require two distinct types of receptors with functionally separate afferent fibers. It is remarkable that so far as they have been adequately studied, all irritants at the carotid gland cause the same general response pattern in the organism: hyperpnea, vasoconstriction and bradycardia. In the present experiments there was frequently a remarkable similarity in respiratory responses to anoxia and hypercapnia in the same animal, before and after vagotomy (fig. 2, A and B; 3, A and B). Cyanides and sulfides were avoided for fear of an irreversible effect on tissue oxidations that would have complicated observations with the iodoacetic acid procedure. But in those experiments wherein there seemed to be no great obstruction in the delicate carotid body circulation, short "blocks" of asphyxial fluid caused abrupt, intense respiratory responses which are scarcely distinguishable from those to cyanide or sulfide reported in other experiments (compare figs. 4 and 5 with cyanide or sulfide responses reproduced in other papers: Winder, Winder and Gesell, 1933; Winder and Winder, 1933; Winder, 1933). It was pointed out earlier (Winder and Winder, 1933) that cyanide and sulfide responses cannot be distinguished from one another. It is believed, then, that subserving the respiratory reflex there is either a common set of receptors and afferent fibers affected by various chemical conditions of the blood; or alternatively, two different sets with very similar central connections, normally subserving the anoxic and the hypercapnic reflexes, respectively. Bernthal (personal communication) has observed that the initial blood-flow changes in the dog's limb caused by various chemical changes at the carotid gland which are now known to increase the receptor discharge, are remarkably similar. Consequently, our belief concerning the respiratory reflex may be applied on the basis of Bernthal's work for the vasomotor reflex also. Further, Samaan and Stella (1935) observed only one type of impulse in the "sinus" nerve in response to chemical stimulation, whether anoxemia, hypercapnia, cyanide or nicotin was used. Zotterman (1935) pointed out that such impulses are propagated by small medullated fibers. These considerations suggest that if the respiratory, vasomotor, and it may be added, the cardiac reflexes, each responding to its various stimuli,

are not subserved by a single common set of fibers, the alternatively multiple sets must be of very similar type.

Even were respiratory responses to anoxia and hypercapnia at the carotid gland subserved by a common set of receptors, it would be surprising to find that the local excitatory process is set up in an identical manner by such differing conditions as deprivation of oxygen, overtension of carbon dioxide, and the many drugs and chemicals. In fact, Bernthal (personal communication), using prolonged exposures, has noted that the response to local anoxemia may build up gradually to a maximum whereas the response to local hypercapnia may reach its maximum sooner. The former reminds one of the gradual accumulation of lactic acid in blood and tissues (Gesell, Krueger, Gorham and Bernthal, 1930; Gesell, Krueger, Nicholson, Brassfield and Pelecovich, 1932), and the commonly observed gradual augmentation of breathing, during low alveolar oxygen administration.

SUMMARY

A study of anoxic, hypercapnic and asphyxic stimulation of the carotid gland before and after poisoning with mono-iodoacetic acid leads to the following observations: There is a progressive increase in ventilation of the animal during the onset of poisoning of the carotid segments, apparently due to an action at the carotid gland. Concentrations of the poison which abolish the reflex anoxic augmentation of ventilation or convert it to a late depression may affect the pressoreceptor reflexes very little. Under certain conditions the anoxic response may be thus abolished or converted while the hypercapnic response persists strongly. Hypercapnia appears able to stimulate the poisoned receptors under a degree of anoxia which by itself is incapable of stimulating.

It is concluded that anoxic stimulation of chemoreceptors of the carotid gland is dependent upon glycolytic processes, and that hypercapnic stimulation may be at least largely independent of such processes; that glycolysis is probably an actual link in setting up the local excitation by anoxia, rather than merely a necessary source of energy during the anoxia; and that the required part of that link is to be found in the formation of intermediary or final acid products of glycolysis.

The question is brought up concerning whether anoxemia and hypercapnia, each with its own acid mechanism, actually act upon a single set of receptors, or require two distinct sets.

REFERENCES

- BARRENSCHEEN, H. K. AND K. BRAUN. *Biochem. Ztschr.* **231**: 144, 1931.
BRONK, D. W. AND G. STELLA. *This Journal* **110**: 708, 1935.
DOBROWOLSKI, N. *Arch. internat. de pharmacodyn. et de therap.* **45**: 428, 1933.
FIELD, J. AND E. S. PEEKE. *Proc. Soc. Exper. Biol. and Med.* **29**: 358, 1931.

- GESELL, R. *Physiol. Rev.* **5**:551, 1925; *Ergebn. d. Physiol.* **28**:346, 1929.
- GESELL, R., H. KRUEGER, G. GORHAM AND T. BERNTHAL. *This Journal* **94**:300, 1930.
- GESELL, R., H. KRUEGER, H. NICHOLSON, C. BRASSFIELD AND M. PELECOVICH. *This Journal* **100**: 202, 1932.
- GESELL, R. AND C. MOYER. *Quart. J. Exper. Physiol.* **24**: 331, 1935a; *Ibid.* **25**: 13, 1935b.
- HAARMAN, W. *Biochem. Ztschr.* **256**:326, 1932.
- HALDI, J. *This Journal* **101**:469, 1932.
- HALL, V. E. AND J. FIELD. *Proc. Soc. Exper. Biol. and Med.* **29**:360, 1931.
- HENDERSON, Y. AND L. A. GREENBERG. *This Journal* **107**:37, 1934.
- IRVING, J. T. *J. Physiol.* **80**:360, 1934.
- LEDEBUR, J. *Pflüger's Arch.* **230**:229, 1932.
- LUNDGAARD, E. *Biochem. Ztschr.* **217**: 162, 1930a; *Ibid.* **220**: 1, 1930b; *Ibid.* **220**: 8, 1930c; *Ibid.* **227**: 51, 1930d.
- MARENZI, A. D. AND W. A. MUNDT. *Compt. rend. Soc. de biol.* **108**:127, 1931.
- NEUSS, F. *Arch. f. exper. Path. u. Pharmacol.* **160**: 551, 1931.
- SAMAAN, A. AND G. STELLA. *J. Physiol.* **85**:7P, 1935.
- SCHMITT, E. *Ztschr. f. Biol.* **96**:467, 1935.
- UEDA, J. *Jap. J. M. Sc., Tr., iv, Pharmacol.* **8**: 45, 1934.
- WINDER, C. V. *This Journal* **106**:28, 1933.
- Ibid.* (preceding paper).
- WINDER, C. V. AND H. O. WINDER. *This Journal* **105**:337, 1933.
- WINDER, C. V., H. O. WINDER AND R. GESELL. *This Journal* **105**:311, 1933.
- ZOTTERMAN, Y. *Skandinav. Arch. f. Physiol.* **72**:73, 1935.

